Figure 5. Endorepellin is counter-adhesive for endothelial, fibrosarcoma and colon carcinoma cells. Figure 5**a**, HUVEC adhesion to increasing concentrations of various substrata including fibronectin (✯), BSA (ỉ), or endorepellin (Θ). For each point, 5 X10^4 cells are seeded on the various substrata. After 1 h, adherent cells are washed, stained with crystal violet and solubilized in O.1% Triton X-100, and absorbance monitored at 600 nm. The number of attached cells is proportional to the absorbance. About 80% of the total cells are attached in the plateau region of the fibronectin curve. The values represent the mean ±SE (n=4). Figure 5**b**, Gallery of light micrographs of crystal violet-stained HUVECs adhered to 50 nM fibronectin following incubation for 1 hr with endorepellin at the indicated concentrations. Scale bar, 125 μm. Figure 5**c** and Figure 5**d**, Adhesion assays for HT1080 fibrosarcoma and WiDr colon carcinoma cells, respectively, on fibronectin (✯), BSA (ỉ), or endorepellin (Θ) substrata. The conditions are identical to those described in panel a. The values represent the mean ±SE (n=4). Figure 5**e** and Figure 5**f**, Displacement curves employing increasing concentration of either endorepellin or endostatin, respectively. The calculated IC_{50} for HT1080 and WiDr was 110 and 40 nM, respectively. The values represent the mean ±SE (n=4).

Remarks

Claims 1-15 are pending in the application. Claim 15 is under examination, claims 1-14 having been previously withdrawn from consideration.

Claim 16 has been added herein. Claim 16 depends from claim 15 and introduces no new subject matter. Support for newly added claim 16 can be found throughout the document, but particularly at page 12, lines 5-22.

The Description of the Drawings has been amended. No new matter is introduced by way of this amendment and support for this amendment is found throughout the specification as filed, including the figures and the description of the results on pages 18-22.

Objection to Drawings

The Examiner has objected to the drawings as failing to comply with 37 CFR 1.84(p)(5), asserting that 1a-1g, 2a-2d, 3a-3d, 4a-4b, and 5a-5f, are not mentioned in the description. In a good faith effort to expedite prosecution of the application the five composite figure description paragraphs as filed under the section entitled "**DESCRIPTION OF THE DRAWINGS**", have been replaced with five new paragraphs. The word "Figure" and the figure number have been added in each case where only a letter was used previously to describe one of a group of figures. A marked up copy of the changes to the five paragraphs is provided as Appendix A.

Rejection of Claim 15 under 35 U.S.C. § 112, second paragraph

Claim 15 stands rejected as allegedly being indefinite. In the opinion of the Examiner, it is unclear as to which "fragments," derivatives," and "analogs," are encompassed by the claim. Applicant respectfully submits that the terms "fragments," derivatives," and "analogs," are definite and that the specification as filed does allow determination of the metes and bounds of the terms. Applicant further asserts that the terms "fragments," derivatives," and "analogs" were well known to those of ordinary skill in the art at the time the application was filed and that their use is consistent with the art-recognized definitions of "fragments," derivatives," and "analogs".

Applicant asserts that the terms "fragments," derivatives," and "analogs" are not indefinite because they were adequately described throughout the specification as filed. For example, at page 12 of the specification, beginning at line 4, is the section entitled "Endorepellin, analogs and fragments thereof," which describes the basis for the use of the terms "fragments," derivatives," and "analogs" relative to endorepellin. At page 12, lines 6-9, it is stated ". . . endorepellin also includes fragments of the 705 amino acid protein and modified proteins and peptides that have a substantially similar amino acid sequence, and which are capable of inhibiting angiogenesis." That phrase describes both physical and biological characteristics of a fragment of endorepellin, namely, a substantially similar amino acid sequence to the parent sequence and the ability to inhibit angiogenesis. That is, the phrase describes a fragment of the 750 amino acid protein and peptides having a substantially similar amino acid sequence (physical characteristics) and the functional

characteristic of biological activity. Both "substantial sequence homology" and "angiogenesis-inhibiting activity" are defined at page 3, lines 4-8.

Applicant respectfully points out that the specification as filed also discloses specific fragments of endorepellin. For example, the specification provides data comparing the activity of the full length sequence of endorepellin, comprising amino acids 3687-4391 (domain V) of the perlecan sequence (Murdoch et al., 1992, J. Biol. Chem., 267:12:8544-8557; copy enclosed), to seven different fragments or deletion mutants of the invention (Figures 1f, 1g). The full length sequence of perlecan, as well as the description of its domains, were known to those of skill in the art at the time the specification was filed (Murdoch et al., 1992, J. Biol. Chem., 267:12:8544-8557).

The disclosure demonstrates that two of the deletion fragments of endorepellin, Δ1 and Δ5, comprising fragments a.a. 3687-4181 and a.a. 3927-4181, respectively, possess endostatin binding activity (page 19, lines 11-24; Fig. 1f, Fig. 1g.). Although only fragment names and lengths based on residue position are disclosed in the specification, the entire gene and protein sequences for perlecan and its domain V (referred to in the specification as filed as endorepellin) are provided in Figure 2 of reference number 5 of the specification (Murdoch et al., 1992, J. Biol. Chem., 267:12:8544-8557). The endorepellin fragment names and lengths disclosed in the specification are based on the sequence published in Murdoch et al. Thus, sequence information for endorepellin was available to those of ordinary skill in the art at the time the application was filed. Armed with this information and with specific biologically active fragments of endorepellin as disclosed in the specification, one of skill in the art would understand what is meant by a "fragment" of endorepellin.

The invention also provides for a 25 kDa cleavage fragment of endorepellin (81 kDa) which is reactive with the anti-His6 antibody directed against endorepellin (page 24, lines 4-7).

Thus, Applicant respectfully submits that ample support is provided for use of the term "fragment" and that armed with the disclosure of the specification as filed, one of ordinary skill in the art would be able to determine the metes and bounds of the term "fragment" as applied to endorepellin.

The above remarks and examples regarding "fragments" apply with equal weight to the terms "analog" and "derivative." Applicant asserts that the terms "analog" and "derivative" are not indefinite because they are amply supported by the specification as filed and that the terms "analog" and "derivative" were commonly used in the art at the time the specification was filed. For example, it was known to one of ordinary skill in the art at the time the specification was filed that an "analog" is a compound that is similar in structure and function, but not identical in composition to a parent compound (*Concise Dictionary of Biomedicine and Molecular Biology*, 1996, CRC Press, New York; copy enclosed). Further, "analog" is defined at page 2, line 31 to page 3, line 3 of the specification as a derivative or modification of the native sequence of endorepellin, including single or multiple amino acid substitutions, additions, or deletions which do not destroy angiogenesis-inhibiting activity. Thus, the definition of "analog" in the specification as filed encompasses both physical and functional characteristics. Specifically, the description details general and specific physical changes and modifications of a peptide of the invention and it also provides for biological function, e.g., that biological activity must be maintained. Therefore, one of ordinary skill in the art is provided with ample information from the disclosure and from that which was known in the art at the time the specification was filed to understand the term an "analog" of endorepellin.

The term "derivative" was in common use and known to those of skill in the art at the time the specification was filed. For example, "derivative" was defined as a compound obtained by modification of a parent compound, particularly chemical modification (*Dictionary of Biochemistry and Molecular Biology*, 2nd ed., 1989, John Wiley & Sons, Inc., New York; copy enclosed). A peptide may be subjected to many known chemical modifications which are known to those of ordinary skill in the art. "Derivatives" are supported at page 3, lines 3-5 and at page 12, lines 25-32. Applicant further asserts that one of ordinary skill in the art at the time the specification was filed would have known of the many methods which could be used to modify a peptide to obtain a derivative as disclosed in the application as filed.

Applicant respectfully submits that the specification as filed provides adequate disclosure for one of ordinary skill in the art to determine the metes and bounds of the terms "fragments," derivatives," and "analogs" of endorepellin as described in the specification

and used in claim 15. Applicant requests withdrawal of the 35 U.S.C. § 112, second paragraph, rejection as applied to these terms.

The Examiner also asserts at page 3 of the Office Action that the metes and bounds of the term "endorepellin" cannot be determined because the amino acid sequence and length is not specifically disclosed in the specification. It is the opinion of the Examiner that the specification discloses that endorepellin is the C-terminal portion of perlecan or domain V of perlecan (citing page 2, lines 4-7 of the application) wherein the meaning of the term encompasses an amino acid that is between 210-705 amino acids in length. The recitation at page 2 of the specification referred to by the Examiner does not refer to the size of endorepellin. Applicant assumes that there was a typographical error in the office action, because the recitation of endorepellin size is found in the specification at page 12, lines 4-7. and Applicant responds accordingly. Applicant asserts that the term endorepellin is definite because the specification as filed does provide adequate disclosure to determine the metes and bounds of the term.

Applicant respectfully submits that there was adequate disclosure in the specification as filed and that there was adequate information known to those of skill in the art at the time the specification was filed to determine the metes and bounds of the term "endorepellin," including adequate sequence information. As discussed above, Figures 1f and 1g disclose use of various amino acid fragments and their locations, based on residue number, in endorepellin and perlecan. In fact, domain V, or endorepellin, comprising amino acids 3687-4391 of the perlecan sequence, is provided in both Figures 1f and 1g of the specification as filed. That domain and the fragments shown in Figures 1f and 1g, demonstrate that an active endorepellin, or fragment thereof, comprises a peptide of about 210 to 705 amino acids in length. For example, fragments a.a. 3687-4181 (494 amino acids in length) and a.a. 3927-4181 (254 amino acids in length) of domain V (a.a. 3687-4391) retain functional activity at a level comparable to full length endorepellin (Figures 1f and 1g). Thus, one of ordinary skill in the art would understand that endorepellin as described in the specification comprises a peptide of about 210 to 705 amino acids. Furthermore, the nucleic acid and amino acid sequences were provided in Figure 2 of reference 5 of the specification (Murdoch et al., 1992, J. Biol. Chem., 267:12:8544-8557), and were known to those of ordinary skill in the art at the time the specification was filed.

Murdoch et al. also presented a molecular model of all the domains of perlecan in Figure 3 (1992, J. Biol. Chem., 267:12:8544-8557). In addition, Murdoch et al. described several characteristics of domain V, including that it is a 705 amino acid terminal module of perlecan, at page 8550, column 2, to page 8551, column 1, last paragraph. The endorepellin peptide and fragments disclosed in the specification as filed are based on the sequence published by Murdoch et al.

Based on the foregoing, Applicant respectfully submits that "endorepellin" is not indefinite. Armed with the information provided in the specification and with the information available in the prior art at the time the specification was filed, one of ordinary skill in the art would be able to determine the metes and bounds of "endorepellin."

Applicant respectfully requests reconsideration and withdrawal of the rejection of claim 15 under 35 U.S.C. 112, second paragraph, for indefiniteness.

Rejection of Claim 15 under 35 U.S.C. § 112, first paragraph, written description

Claim 15 stands rejected for lack of written description. At page 3 of the office action, the Examiner asserts that the written description in the specification is limited to endorepellin protein and is therefore not commensurate in scope with the recitation of fragments, derivatives, or analogs of endorepellin in claim 15. The Examiner cites *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111 (Fed. Cir. 1991) to support his argument. The Examiner does admit at page 5 of the Office Action that ". . . endorepellin protein, in so far as it reads on a protein that is between 210 and 705 amino acids in size," meets the written description requirement of 35 U.S.C. § 112, first paragraph. To this end, Applicant has added a new pharmaceutical composition claim specifically reciting an endorepellin protein that is between 210 and 705 amino acids in size.

Applicant respectfully submits that the specification as filed provides adequate written description for endorepellin fragments, derivatives, or analogs. The above remarks regarding 35 U.S.C. § 112, second paragraph support for endorepellin fragments, derivatives, or analogs apply to the written description requirement of 35 U.S.C. § 112, first paragraph as well.

As outlined in MPEP § 2163, a description need only describe in detail that which is new or not conventional. See *Hybritech v. Monoclonal Antibodies*, 802 F.2d 1367, 1384,

231 USPQ 81, 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805.

Preliminarily, it is well-settled law that the written description requirement is viewed in light of the state of the art and skill of the practitioner at the time the application was filed. In *Vas-Cath, Inc. v. Mahurkar,* 19 USPQ2d 1111 (Fed. Cir. 1991), the Court of Appeals for the Federal Circuit traced the development of the written description requirement under 35 U.S.C. §112, first paragraph. The *Vas-Cath* Court, in a unanimous opinion, noted approvingly that in a written description analysis, "[t]he primary concern is factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure." *Vas-Cath*, 19 USPQ2d at 116 (quoting *In re Wertheim*, 191 USPQ 90, 96 (C.C.P.A. 1976)). After discussing the policy reasons underlying the requirement, the Court set forth the standard for the written description requirement:

> The purpose of the "written description" requirement is broader than to merely explain how to "make and use"; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. . . . The test for sufficiency of support in a parent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter."

Vas-Cath, 19 USPQ2d at 1117 (emphasis added) (quoting *Ralston Purina Co. v. Far-Mar-Co., Inc.*, 227 USPQ 177, 179 (Fed. Cir. 1985)). *Accord University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997). Therefore, it is well-settled that the knowledge of those skilled in the art informs the written description inquiry.

In determining the sufficiency of support in a disclosure with respect to the written description requirement, "it is not necessary that the application describe the claimed invention in *ipsis verbis*; all that is required is that it reasonably convey to persons skilled in the art that, as of the filing date thereof, the inventor had possession of the subject matter later claimed by him." *In re Edwards*, 196 USPQ 465, 467 (C.C.P.A. 1978) (citing *In re Lukach*, 169 USPQ 795 (C.C.P.A. 1971); *In re Driscoll*, 195 USPQ 434 (C.C.P.A. 1977)).

More recently, the Court of Appeals for the Federal Circuit, in *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983), citing *In re Edwards*, emphasized:

> The test for determining compliance with the written description requirement is whether the disclosure of the application as originally filed reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter, rather than the presence or absence of literal support in the specification for the claim language. (Emphasis added).

More recently, in *In re Alton*, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996), the court of Appeals for the Federal Circuit pointed out that literal support is not required in order to satisfy the written description requirement:

> If a person ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate written description requirement is met. For example, in *Ralston Purina Co. v. Far-Mor-Co., Inc.*, 227 USPQ 177, 180 (Fed. Cir. 1985), the trial court admitted expert testimony about known industry standards regarding temperature and pressure in "the art of both farinaceous and proteinaceous vegetable materials." The effect of the testimony was to expand the breadth of the actual written description since it was apparent that the inventor possessed such knowledge of industry standards of temperature and pressure at the time the original application was filed. (Emphasis added).

Therefore, it is clear that the invention need not be described in *ipsis verbis, i.e.*, literally, for purposes of the written description requirement under 35 U.S.C. §112, first paragraph. Rather, what is needed is that the skilled artisan understand, based upon the disclosure in the specification as filed and the knowledge imputed to the skilled artisan at the time the specification was filed, that the inventor had possession of the claimed subject matter.

Applicant respectfully submits that one skilled in the art, upon reading the specification as filed, would have understood that the invention encompassed an endorepellin protein and fragments, derivatives, and analogs of endorepellin. As described above, endorepellin protein and endorepellin fragments, derivatives and analogs are supported in the specification as filed. At page 5 the Examiner admits that "Support for fragments, derivatives, and or analogs can be found on page 2 lines 31-33 and page 3 lines

1-3." The specification provides the entire domain V of perlecan as well as five specific fragments of domain V (endorepellin), including their location within perlecan, in Figures 1f and 1g, and as described above, the nucleic acid and amino acid sequences for endorepellin were known in the art at the time the specification was filed. The specification provides relevant molecular and biochemical methods for preparing endorepellin protein and endorepellin fragments, derivatives, and analogs, as well as multiple assays with which to test the ability of endorepellin protein and endorepellin fragments, derivatives, and analogs to inhibit angiogenesis (see pages 3-25).

Based upon the disclosure in the specification as filed and the knowledge imputed to the skilled artisan at the time, a skilled artisan would indeed understand that the applicant had possession of the claimed subject matter.

The Examiner also asserts at page 4 of the Office Action that *Fiers v. Revel*, 25 USPQ2d 1601 at 1606, and *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 are applicable because no amino acid sequence is provided in the specification. Applicant respectfully submits that *Fiers* and *Amgen* are inapplicable because, as described above, sequence and fragment information for endorepellin are provided in the specification or was known to those of skill in the art. The Examiner further asserts that *The Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412) is applicable because a generic statement does not provide an adequate written description when only functional activity is described.

Applicant respectfully submits that *Eli Lilly* is inapplicable because more than functional activity is provided for endorepellin fragments, derivatives and analogs. As discussed in detail above, ample support is provided for specific endorepellin fragments in Figures 1f and 1g, including references to specific lengths and positions of the fragments within endorepellin. In addition, the sequence of perlecan and the existence of its various domains was known to those of skill in the art at the time the specification was filed. Furthermore, *Eli Lilly, Fiers,* and *Amgen* are all inapplicable because each case is drawn to DNA and not to proteins or to protein fragments, derivatives, or analogs.

At page 4 of the Office Action, the Examiner alleges that "allelic variants" lacks adequate written description and further alleges that the amino acid sequence for such variants is required. Applicant respectfully points out that "allelic variants" are not

discussed or recited in the specification. Even if allelic variations did exist for the perlecan gene, those variations are not relevant to this application, where the sequence of endorepellin protein, fragments, derivatives, or analogs thereof, are provided. As discussed above, sequence information was provided in the specification as filed for endorepellin protein, "fragments," "derivatives,' or "analogs thereof," or was known to those of skill in the art at the time the specification was filed. As discussed above, Figures 1f and 1g of the specification describe specific fragment lengths and positions of the amino acid residues for the amino and carboxy termini of each fragment for endorepellin and fragments of endorepellin disclosed in the invention. Also as described above, the nucleic acid and amino acid sequences for perlecan and domain V (endorepellin) of perlecan were known to those of ordinary skill in the art (Murdoch et al.) at the time the specification was filed.

In view of the present specification as filed and the prior art usage as discussed above, one of ordinary skill in the art would readily understand the definition and scope of the claims as filed. Applicant respectfully requests reconsideration and withdrawal of the 35 U.S.C. § 112, first paragraph, written description rejection.

Rejection of Claim 15 under 35 U.S.C. § 112, first paragraph, enablement

At page 5 of the Office Action, the Examiner has rejected claim 15 for lack of enablement. The Examiner asserts, inter alia, that the art is unpredictable, the specification has not provided an enabling disclosure in the form of a working model, and that because of uncertainty of the capabilities of the proteins to function in vivo, that it would require undue experimentation to practice the invention as claimed. At page 6 of the Office Action, the Examiner asserts that Dermer (Bio/Technology 1994:12:320) teaches that in vitro representations of malignancy or cancer have profoundly different characteristics from human disease.

The Examiner alleges at page 7 of the Office Action that Freshney (*Culture of Animal Cells, A Manual of Basic Techniques*, Alan R. Liss, Inc., 1983, New York, p. 4) teaches that there are many differences between cultured cells and their in vivo counterparts, wherein the difference stems from the dissociation of cells from a three-dimensional geometry to a two-dimensional substrata. The Examiner also asserts at page 7 that the specification does not teach the effect of endorepellin in vivo and its ability to function as

claimed as a pharmaceutical composition. In the view of the Examiner, the specification does not teach the use of endorepellin in vivo because there is a lack of enabling disclosure and that a working example is not provided. Applicant respectfully submits that these allegations are inapplicable because they do not address angiogenesis. Claim 15 is enabled by the specification, based on the following reasons.

A specification which discloses how to make and use a claimed invention is presumed to comply with the first paragraph of 35 U.S.C. § 112, unless there is a reason to doubt the objective truth of the specification. *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971). The initial burden of establishing a basis for denying patentability to a claimed invention therefore rests upon the examiner. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Thorpe*, 777 F.2d 695, 227 USPQ 964 (Fed. Cir. 1985); *In re Piasecki*, 745 F.2d 1468, 223 USPQ 785 (Fed. Cir. 1984). Here, the present specification clearly discloses how to make and use the claimed endorepellin peptide and fragments, derivatives, and analogs thereof, and how to use them in vitro and in vivo, and the Examiner has failed to rebut the assertions made therein.

It is well-settled that an applicant need not have actually reduced the invention to practice prior to filing in order to satisfy the enablement requirement under 35 U.S.C. §112, first paragraph. MPEP §2164.02 (citing *Gould v. Quigg*, 822 F.2d 1074 (Fed. Cir. 1987)). Indeed, the invention need not contain a single example if the invention is otherwise disclosed in such manner that one skilled in the art will be able to practice it without an undue amount of experimentation (*In re Borkowski*, 422 F.2d at 908), and "representative samples are not required by the statute and are not an end in themselves" (*In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970)). Thus, 35 U.S.C. § 112, first paragraph, enablement does not require any working examples.

The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. MPEP §2164.01 (citing *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976)). The fact that experimentation may be complex does not necessarily make it undue if the art typically engages in such experimentation. *Id.* Further, the specification need not disclose what is well known to those skilled in the art and preferably omits that which is well-known to those skilled in the art and is already available to the public. MPEP §2164.05(a) (citing *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir.

1991)). Enablement does not require a working example. Experimentation is allowed, so long as it is not undue.

Dermer and Freshney are inapplicable because neither is relevant to enablement for a protein, or its fragments, derivatives, or analogs, which inhibits angiogenesis. Dermer and Freshney have no relevance to angiogenesis. Dermer recites potential in vitro and in vivo differences which may arise when treating malignancies or cancer. While angiogenesis may be a component of tumor growth, the utility of the claimed pharmaceutical composition is not just for treating cancer per se. In addition, Dermer does not show that the results of in vitro angiogenesis assays, such as those described in the specification, cannot be extrapolated to the use of the anti-angiogenesis agent in vivo. Thus, Dermer is not applicable to the present invention, which describes using a protein or its fragments, derivatives, or analogs to inhibit angiogenesis.

Freshney's teaching regarding cells being different when grown on a two-dimensional substrate instead of in a three-dimensional environment is inapplicable here for several reasons. Examiner has not demonstrated that the angiogenesis assays described in the specification would suffer from these same deficiencies attributed to the 2-D assays by Freshney. Indeed those purported deficiencies would not be expected for the assays of the specification, which are #-D, not 2-D, assays. For example, the specification describes a migration assay which demonstrates the ability of endothelial cells to migrate through pores in a membrane (page 19, line 26 to page 20, line 17; Figures 2a-2c). The cells are free to move in three dimensions. The specification also describes the use of in vitro three-dimensional vessel/tube formation assays, which mimic blood vessel formation in vivo, to test the effect of endorepellin on angiogenesis (page 20, line 25 to page 21, line 5; Figures 3a-3d). This also is a 3-D assay. The in vivo chicken chorioallantoic membrane (CAM) assay described and used in the specification to test the effect of endorepellin on endothelial cell migration and blood vessel formation in vivo is also a 3-D assay(page 20, lines 18-24; Figure 2d). Thus, Freshney's discussion of the shortcomings of two-dimensional in vitro models is not applicable to the present specification, which utilizes angiogenesis assays which are conducted in three, not two, dimensions.

Examiner alleges at page 7 that the specification has failed to demonstrate endorepellin's anti-angiogenic use in vivo. This is incorrect. The CAM assay is an in vivo

assay (page 20, lines 18-24; Figure 2d). The CAM assay is an accepted in vivo animal model of angiogenesis. It has been accepted by the USPTO. The CAM assay was utilized in U.S. Patent No. 6,284,726 (submitted herewith) for demonstrating the anti-angiogenic activity of certain peptide analogs of high molecular weight kininogen domain 5. See column 11, lines 50-51, of U.S. Patent No. 6,284,726: "[t]he effect of the HK domain 5 peptides on cytokine-stimulated angiogenesis in vivo. . .,". Also Figures 1A to 1D for the results of that CAM assay. The undersigned was attorney of record in Patent 6,284,726. Claims directed to pharmaceutical compositions and methods of inhibiting angiogenesis issued on the basis of the data in the specification. No additional data of anti-angiogenic effect was submitted during prosecution.

At the time the specification was filed, it was known to those of skill in the art that proteins which inhibit angiogenesis in vitro, in models such as the three-dimensional models described in the specification, would also inhibit angiogenesis in vivo. References cited in the specification demonstrated the effects of angiogenesis-modulating proteins such as perlecan and endostatin using in vitro and in vivo assays (Aviezer et al., 1994, Cell 79:6:1005-1013; Nugent et al., 2000, Proc. Natl. Acad. Sci. USA 97:6722-6727; O'Reilly et al., 1997, Cell 88:277-285; Yamaguchi et al., 1999, EMBO J. 18:4414-4423).

Further evidence of the correlation between the in vitro and in vivo anti-angiogenic effects of endorepellin peptides is provided by Mongiat et al. (2003, J. Biol. Chem. 278:6:4238-4239). In Mongiat et al., Matrigel® plugs containing fibroblast growth factor-2, in the presence or absence of endorepellin, were injected subcutaneously into mice. It was found that endorepellin caused a marked inhibition of neovascularization within and around the Matrigel® plug (Figures 5a to 5d).

In sum, Applicant respectfully submits that claim 15 is supported by the disclosure provided in the specification as filed. Therefore, undue experimentation would not be required of a skilled artisan to make and/or use the full scope of the invention in vivo as recited in claim 15. Given the advanced state of the relevant art, the ample disclosure, and the extensive reduction to practice provided in the specification as filed, claim 15 is enabled and this requirement of 35 U.S.C. § 112, first paragraph, has been satisfied. Thus, Applicant respectfully requests that the rejection under 35 U.S.C. § 112, first paragraph, be reconsidered and withdrawn.

Conclusion

Based on the foregoing, all claims under review are believed to be in condition for allowance. An early and favorable action toward that end is earnestly solicited.

Respectfully submitted,

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Appendix A- Marked-up copy of amendments to "Description of the Drawings"

Figure 1. Perlecan domain V (endorepellin) binds to the anti-angiogenic factor endostatin. Figure 1**a**, Agarose gel showing the 1.7 kb cDNA strongly interacting with endorepellin, obtained from the BglII digestion of clone A3. Complete sequence of A3 clone revealed the C terminus of type XVIII collagen. Figure 1**b**, Schematic representation of the human α chain of type XVIII collagen. The triple-helical and non-triple helical domains are indicated by rods and blue boxes, respectively. The C-terminal endostatin fragment is highlighted in orange. The beginning of the clone A3 sequence is shown (NCBI accession # AF018082). Figure 1**c**, Growth and β-galactosidase activity triggered by the interaction of endorepellin with collagen type XVIII fragment compared to the positive (p53 and T-antigen) and negative control (lamin and T-antigen). Figure 1**d**, Co-immunoprecipitation of collagen XVIII (clone A3) and endorepellin following *in vitro* transcription/translation using [^{35}S]methionine as the labeled precursor. Endorepellin (lane 1) and collagen XVIII (lane 2) are mixed in equimolar amounts and co-immunoprecipitated with either anti-hemagglutinin (α-HA) (lane 3) or no antibody. Figure 1**e**, Co-immunoprecipitation of endostatin with endorepellin. Domain III (lane 1), endorepellin (lane 2) and endostatin (lane 3) were generated by *in vitro* transcription/translation using [^{35}S]methionine as the labeled precursor. Endostatin was mixed with either domain III (lane 4) or endorepellin (lane 5) and immunoprecipitated with anti-hemagglutinin (α-HA) antibody. Figure 1**f**, Schematic representation of domain V and various deletion mutants. Orange ovals indicate laminin–type G modules (LG), whereas blue rectangles indicate EGF-like (EG) modules. The growth is indicated by semi-quantitative assessment with maximal growth at +++. The numbers within parentheses designate the amino acid position based on the mature protein core. Figure 1**g**, Representative α and β-galactosidase assays of various deletion mutants, as indicated; pGB53/pGADT was the positive control.

Figure 2. Endorepellin is a powerful anti-angiogenic factor. Figure 2**a**, Purification of endorepellin from media conditioned by 293-EBNA cells expressing the 81 kDa endorepellin tagged with His6. Coumassie-stained SDS-PAGE (left) and Western immunoblotting with anti-His6 antibody (right) of negative control media (lanes 1 and 4),

flow through (lanes 2 and 5), and 250 mM imidazole eluate (lanes 3 and 6). Figure 2**b** and Figure 2**c**, HUVEC migration assays through fibrillar collagen using 10 ng/ml VEGF as a chemotactic inducer and preincubation the HUVECs for 30 min with various concentrations of endostatin (ES) and endorepellin (ER). Serum free medium (SFM). Figure 2**d**, CAM assays three days after the application of sponges containing VEGF (1 ng), VEGF (1 ng)+ endorepellin (400 ng), or buffer alone. Scale bar, 1 mm.

Figure 3. Endorepellin, but not endostatin, blocks endothelial tube formation induced by fibrillar collagen. Figure 3**a**, Figure 3**b**, Figure 3**c**, and Figure 3[-]**d**, Gallery of light micrographs capturing the time course production of HUVEC tube-like formation in fibrillar collagen containing either buffer (Control), endorepellin, endostatin, or both at the designated concentrations. In this assay, 4 X 10^5 cells are incubated for 24 hr and pictures are taken at various intervals as indicated in the top margins. Scale bar, 250 μm.

Figure 4. Biological consequences of endostatin/endorepellin interaction. Figure 4**a** and Figure 4**b**, HUVEC migration assays through fibrillar collagen using 10 ng/ml VEGF as a chemotactic inducer and preincubation the HUVECs for 30 min with various concentrations of endostatin (ES), endorepellin (ER), or various combinations as indicated. The values are presented as the percentage of maximal stimulation induced by VEGF alone, arbitrarily set at 100%. [Panel] Figure 4a is the summary of three independent experiments run in quadruplicates, mean ±SE. The values in [panel] Figure 4b derive from an additional experiment run in quadruplicate, mean ±SE. Serum free medium (SFM).

Figure 5. Endorepellin is counter-adhesive for endothelial, fibrosarcoma and colon carcinoma cells. Figure 5**a**, HUVEC adhesion to increasing concentrations of various substrata including fibronectin (✯), BSA (ɪ̇), or endorepellin (Θ). For each point, 5 X10^4 cells are seeded on the various substrata. After 1 h, adherent cells are washed, stained with crystal violet and solubilized in O.1% Triton X-100, and absorbance monitored at 600 nm. The number of attached cells is proportional to the absorbance. About 80% of the total cells are attached in the plateau region of the fibronectin curve. The values represent the mean ±SE (n=4). Figure 5**b**, Gallery of light micrographs of crystal violet-stained HUVECs adhered to 50 nM fibronectin following incubation for 1 hr with endorepellin at the

indicated concentrations. Scale bar, 125 μm. Figure 5**c** and Figure 5**d**, Adhesion assays for HT1080 fibrosarcoma and WiDr colon carcinoma cells, respectively, on fibronectin (✯), BSA (ỉ), or endorepellin (Θ) substrata. The conditions are identical to those described in panel a. The values represent the mean ±SE (n=4). Figure 5**e** and Figure 5**f**, Displacement curves employing increasing concentration of either endorepellin or endostatin, respectively. The calculated IC_{50} for HT1080 and WiDr was 110 and 40 nM, respectively. The values represent the mean ±SE (n=4).

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BIOMEDICINE AND MOLECULAR BIOLOGY

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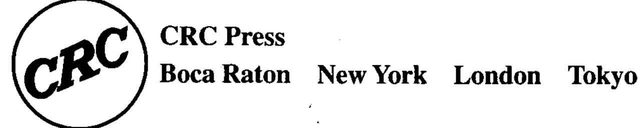

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Anabolism A biosynthetic process by which simple substances are converted into more complex compounds.

Anacidity The lack of gastric hydrochloric acid.

Anacin A trade name for a combination drug containing aspirin and caffeine.

Anacin-3 A trade name for acetaminophen, used as an analgesic and antpyretic agent.

Anacystis A genus of cyanobacteria.

Anacobin A trade name for vitamin B_{12} (cyanocobalamin).

Anaerobe An organism capable of growing in the absence of molecular oxygen.

Anaerobic Pertaining to anaerobe.

Anaerobic Digestion The anaerobic breakdown of complex organic materials (e.g., animal and/or plant materials or sewage) to simple substances.

Anaerobic Fermentation Fermentation in the absence of molecular oxygen.

Anaerobic Glycolysis The pathway that converts glucose to lactic acid in the absence of molecular oxygen (also known as glycolysis).

Anaerobic Photosynthetic Bacteria Bacteria that carry out the photosynthetic reactions of photosystem I in the absence of molecular oxygen.

Anaerobic Respiration The energy-yielding metabolic process that uses substances (e.g., fumarate, nitrate, sulfur) other than oxygen as terminal electron acceptors.

Anaerobiospirillum A genus of Gram-negative bacteria (family Bacteroidaceae).

Anaerobiotic Life under anaerobic conditions.

Anaeroplasma A genus of obligately anaerobic, cell-wall-less, sterol-requiring bacteria.

Anaerovibrio A genus of Gram-negative bacteria (family Bacteroidaceae) that occurs in the rumen.

Anafranil A trade name for clomipramine hydrochloride, used as an antidepressant.

Anagrelide (mol wt 256) An antithrombotic agent and pleatlet aggregation inhibitor.

Analbuminemia A metabolic disorder characterized by an impaired synthesis of serum albumin.

Analgesia The relief of pain without loss of consciousness.

Analgesic 1. Relieving pain. 2. A drug that relieves pain.

Analog 1. Chemical compounds that are similar in structure but nonidentical in composition. 2. Structures that are not homologous but have a similar function. 3. Structures that are similar in function and appearance but nonidentical in origin and development.

Analogous Enzyme Variants Enzyme variants that differ significantly in their molecular structures and catalytic properties.

Analogue Variant spelling of analog.

Analytical Biochemistry Biochemistry that deals with the qualitative and quantitative determination of substances in living systems.

Analytical Method Method that deals with the identification and characterization of specific substances (e.g., electrophoresis, analytical centrifugation, and HPLC).

Analytical Ultracentrifuge A high-speed centrifuge, equipped with optical systems, used for analytical analysis (e.g., determination of sedimentation coefficients and molecular weights).

Anamnesis See Anamestic reaction.

Anamnestic Reaction A heightened immunological response to a previously encountered antigen.

Anaphase A stage in mitosis in which the chromatids of each chromosome separate and move to opposite poles.

Anaphoresis The movement of charged particles or molecules toward the anode.

Anaphylactic Hypersensitivity An IgE-mediated type I hypersensitivity that involves the reaction of allergen with IgE-sensitized mast cells leading to mast cell degranulation; release of bioactive amines (e.g., histamine, serotonin); vasodilation; smooth muscle constriction; or acute asthma, bronchospasm, or death in severe cases. It is also known as immediate-type hypersensitivity.

Anaphylactic Response See Anaphylactic hypersensitivity.

Anaphylactic Shock See Anaphylactic hypersensitivity or type I hypersensitivity.

DICTIONARY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

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catalyzes the hydrolysis of DNA. *Abbr* DNase; DNAase.

deoxyribonuclease I A deoxyribonuclease that catalyzes the hydrolysis of DNA to mono- and oligonucleotides consisting of, or terminating in, a 5′-nucleotide. *Abbr* DNase I; DNAase I.

deoxyribonuclease II A deoxyribonuclease that catalyzes the hydrolysis of DNA to mono- and oligonucleotides consisting of, or terminating in, a 3′-nucleotide. *Abbr* DNase II; DNAase II.

deoxyribonucleic acid The nucleic acid (*abbr* DNA) that constitutes the genetic material in most organisms and that is composed of the genes; together with histones it makes up the chromosomes of higher organisms. DNA is a polynucleotide that is characterized by its content of 2-deoxy-D-ribose and the pyrimidines cytosine and thymine. *See also* DNA forms; Watson–Crick model.

deoxyribonucleoprotein A conjugated protein that contains DNA as the nonprotein portion. *Abbr* DNP.

deoxyribonucleoside A nucleoside of 2-deoxy-D-ribose.

deoxyribonucleotide A nucleotide of 2-deoxy-D-ribose.

deoxyribose The five-carbon aldose, 2-deoxy-D-ribose, that is the carbohydrate component of deoxyribonucleic acid. *Abbr* dRib; deRib.

deoxyribose nucleic acid DEOXYRIBONUCLEIC ACID.

deoxyriboside A glycoside of deoxyribose.

deoxyribotide A deoxyribonucleotide.

deoxysugar A monosaccharide in which one or more hydroxyl groups have been replaced by hydrogen atoms.

deoxythymidine THYMIDINE.

deoxythmidylic acid THYMIDYLIC ACID.

deoxyuridine The deoxyribonucleoside of uracil.

deoxyuridylic acid The deoxyribonucleotide of uracil.

depancreatize To surgically remove the pancreas.

dependent form The phosphorylated form of the enzyme glycogen synthase that is a regulatory enzyme for which glucose-6-phosphate is a positive effector. *Abbr* D-form.

dependent variable A quantity that is a mathematical function of one or more independent variables; the value of a dependent variable is fixed once the values for the related independent variables are chosen.

depolarization The elimination of polarization, as that occurring in a muscle or a nerve membrane upon electrical stimulation. A decrease in membrane potential; the membrane potential becomes less negative than it is in the normal resting state.

depolarization fluorescence *See* fluorescence depolarization.

depolymerization The degradation of a polymer to oligomers and/or monomers.

depolymerizing enzyme An enzyme that catalyzes the hydrolysis of a biopolymer to oligomers and/or monomers.

depot fat The fat that is stored in an organism. *Aka* adipose tissue.

deproteinization The removal of protein from a biological sample.

depside A natural or synthetic ester formed by condensation of phenol carboxylic acids; depsides occur in lichens and tannins.

depsipeptide antibiotics A group of peptide-like antibiotics, produced by *Fusaria* fungi. They consist of alternating amino acid and hydroxy acid residues, with the residues being linked by alternating peptide and ester bonds. Depsipeptide antibiotics are frequently cyclic and are then referred to as cyclodepsipeptides, peptolides, or enniatins. Cyclic depsipeptides act as ionophores.

depurination The removal of purines from a nucleic acid.

depyrimidination The removal of pyrimidines from a nucleic acid.

derepression Any modification that eliminates the repression of a gene and permits the synthesis of the gene product. Possible modifications include a decrease in the repressor concentration produced by starving the organism of a required nutrient, a reaction of the inducer with the repressor, a mutation of the regulator gene, or a mutation of the operator gene.

deRib Deoxyribose.

derivative A compound, usually an organic one, that is obtained by modification of a parent compound as a result of one or more chemical reactions.

derivative spectroscopy A method for analyzing spectroscopic measurements by plotting the first-, second-, or higher-order derivatives of a spectrum with respect to the wavelength.

derivatize To synthesize a derivative.

derived carbohydrate A derivative of a simple sugar, such as a sugar acid or an amino sugar.

derived lipid A lipid obtained by hydrolysis of a naturally occurring lipid.

derived protein A product obtained by treatment of a protein with heat, acid, base, enzymes, or other agents. Primary derived proteins, such as proteins and metaproteins, are proteins that have been altered only slightly; secondary derived proteins, such as proteoses and peptones, are proteins that have been altered more extensively.

dermal Of, or pertaining to, the skin, especially the true skin.